A STUDY OF FERTILITY IN THE BLOWFLY, PHORMIA REGINA MEIGEN.

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In the rearing of blowfly cultures for use in the treatment of osteomyelitis, egg masses are occasionally obtained which fail to hatch. Sometimes this becomes widespread enough among the cultures to seriously interfere with consistent treatment of patients in the clinic. Consequently a study of the problem was undertaken* with one of the flies most frequently used in such treatment (*Phormia regina*) in order to determine:

(1) When sperm production begins in adult males,
(2) How long males produce active sperms,
(3) What part foods and underfeeding play in sperm production,
(4) How long the sperms remain viable in the females after copulation.

There is very little information available upon the subject of fertility in male insects, except by inference from hatchings made in connection with studies on egg production. Lowne (1890–1892) observed mature sperms in *Calliphora erythrocephala* within a few hours after emergence. Environmental factors, especially food, temperature and relative humidity (Wardel, 1930) have been shown to be influential in producing fertile eggs. Baumberger (1919) showed the necessity for yeast in the food of Drosophila and Guyenot (1913) found that sterile potato was not sufficient for complete development and reproduction in the same fly. Young and Plough (1920) have shown how temperature could produce sterility in drosophila.

Glaser (1923) states that houseflies need protein and sugar to produce eggs and that very few eggs are laid by females of *Musca domestica* or *Stomoxys calcitrans* unless they have mated. I have likewise observed this to be true for Phormia. The effects of larval feeding, adult feeding, temperature and humidity upon growth and maximum egg production in *Phormia regina* have been summarized by Miller, Doan and Wilson

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(1932) in studies upon this fly in connection with their treatment of osteomyelitis.

Larson and Fischer (1924) observed that one mating of the pea weevil (*Bruchus quadrimaculatus*) was sufficient to fertilize a large number of eggs.

Occurrence of Spermatazoa in *Phormia regina*.

Because of the importance of environmental factors, as shown by all writers, precaution was taken to grow all experimental cultures in cabinets under controlled temperature (28° C.) and relative humidity (60%). The standard foods (water, meat and sugar) were used and varied to meet the conditions of the experiments. Stock cages of flies were started which contained one hundred and fifty males and one hundred and fifty females in each cage.

Flies fed upon a complete diet.—The first experiment had to do with normally reared adults which were maintained upon a diet of sugar, meat and water. A number of males were removed daily for dissection and examined for the presence of spermatozoa. An equal number of females were removed at the same time in order to maintain the original sex ratio. The abdomens were removed from the males and placed in a drop of water on a microscope slide and dissected under a binocular. The last two abdominal segments were cut loose by drawing the point of a needle across the abdomen. This operation usually disclosed the testes in the cup-shaped hinder two segments. The male organs in Phormia closely resemble those of *Calliphora erythrocephala* as described by Lowne (1890–1892). The testes were next taken out, along with as much of the rest of the reproductive organs as possible, mounted in water under a cover-glass and examined with a compound microscope (10X occular, 4 mm. objective). Usually the weight of the cover glass was sufficient to force out the contents of the testes but when not, gentle pressure with a needle point was used. Although the spermatazoa were quite large and active and therefore not easily overlooked, material was sectioned and stained as a check upon this method.

No active sperms were seen in the flies examined during the first 48 hours, but during the third day sperms became abundant and were found so in most of those flies examined up to the twenty-seventh day. There was some variation in the amount of sperms in different individuals and a few flies were entirely infertile. Since these sterile males were found at various times during the experiment, it is probable that they had never developed germ cells. The results indicate that most of the males, however, remained fertile up to the time of their death. Dissection of a few dead flies substantiated this view by revealing, in several cases, sperms that were still capable of movement, though this does not necessarily mean that they were still capable of fertilizing eggs.

This experiment was repeated with another group of flies, extending over a period of 30 days, and almost identical results were obtained.

Flies fed upon water and sugar only.—In Phormia, as in other muscoid flies, few or no eggs are laid without some protein food in the adult stage. Obviously, sufficient food material is not stored by the larvae which develop into females for eggs to form in the adult. This contrasted with the same treatment in the males, gave an interesting comparison.

Experimental cages were started under the same conditions as in the first experiment except that in this case the only food provided was water and cane sugar. Ten males were dissected each day for the first week and five or more each day thereafter until the twenty-eighth day. Two males dissected late on the second day showed some sperms. From the third to the seventh day sperms were fully as abundant in these as they were in flies fed meat as well. From the eighth to the twenty-eighth day there was considerable variation in the amount of sperms in different individuals, but completely infertile males were no more abundant than in flies fed upon a complete diet of sugar, meat and water.

Flies kept entirely without food.—Another experimental cage of flies was kept under the conditions stated above with the exception that no food was given them at any time after emergence. Most of these flies lived 4 days and some lived until the end of the sixth day. Examinations showed that sperms appeared on the third day as usual, and were quite numerous in all flies dissected up to the sixth day. Several individuals found dead upon the sixth day also contained living sperms. The results of this experiment show that, at least during the first few days of adult life, sperm production is independent of the feeding of the males.

The effects of underfeeding in the larval stage.—Underfeeding during the larval stage affects the adult female fly by increasing the preoviposition period, by shortening the length of adult life and, consequently, by decreasing the total number of eggs laid.

To determine the effect of underfeeding upon the males the following experiment was run. A large culture of larvae was started. Four groups of two hundred larvae each were removed from the culture at the end of 36 hours, 52 hours, 76 hours and 96 hours, respectively. Larvae which had fed but 36 hours failed to pupate and eventually died. All others pupated and emerged. Dissection of the males in these groups showed that sperms appeared at about the same time in each, regardless of the length of larval feeding. Some sperms were found at the end of the first day and were abundant by the third day. This indicates that the time and amount of larval feeding do not affect sperm production in male Phormias though they greatly affect oviposition in the females.

Viability of the sperms in the female.—It has been shown by careful counts that in the normal history of a new cage of adult flies many more males than females die during the first two weeks after emergence. It is quite possible, therefore, for a cage that originally held equal numbers of males and females, to contain few or no males after twenty-five to thirty days. In spite of this reduction in proportion of males, cages have produced fertile eggs for as long as sixty days, suggesting the

possibility that the sperms remain viable for some time in the female after copulation.

To test this the males were removed from two cages of flies. One cage was eight days old, the other eighteen. The first cage had laid fertile eggs once before the removal of the males and the second cage had laid fertile eggs six times. In both of these cages, subsequent to the removal of the males, fertile eggs were laid every second day over a period of eleven days. On the thirteenth and fifteenth days no eggs were laid and most of the females died soon after, being twenty-three and thirty-three days old, respectively, which is the average age of a cage. These results would indicate that sperms are viable in the female at least eleven days after copulation.

SUMMARY.

Referring back to the questions stated at the beginning of this article, we find that they are answered as follows:

(1) In the males of *Phormia regina*, sperms are present regularly on the third day and sometimes as early as the first day after emergence.

(2) Adult males continue to produce sperms throughout their life (at least 28 days).

(3) Although both larval and adult feeding affect fertility and oviposition in the female, fertility in the male is not affected by the length of larval feeding nor by the absence of protein from the diet of the adult. Males kept for six days without any food whatsoever, produced sperms normally until death from starvation.

(4) Sperms remain functional in the females for at least eleven days after separation from the males.

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